

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re application of:

NAKAMURA et al.

Art Unit: 1652

Application No.: 10/720,177

Examiner: Delia M. RAMIREZ

Filing Date: November 25, 2003

Attorney Ref. No.: US-110

For: METHOD FOR PRODUCING L-  
GLUTAMINE AND L-  
GLUTAMINE PRODUCING  
BACTERIUM

Confirmation No.: 6388

**BRIEF FOR APPELLANT**

**Mail Stop Appeal Brief - Patents**

Commissioner for Patents

P.O. Box 1450

Alexandria, VA 22313-1450

Sir:

COMES NOW the Appellant to present this Brief in support of the appeal of the rejections of Claims 1, 4, 5, 12-16 and 18-21 in the above-captioned patent application. The Notice of Appeal having been timely filed on February 9, 2009, this Brief is due to be filed on May 9, 2009, with a one-month extension of time.

It is not believed that extensions of time are required, beyond those that may otherwise be provided for in accompanying documents. If, however, additional extensions of time are necessary to prevent abandonment of this application or dismissal of this appeal, then such extensions of time are hereby petitioned under 37 C.F.R. § 1.136(a), and the Commissioner is hereby authorized to charge fees necessitated by this paper, and to credit all refunds and overpayments, to the credit card authorized on the attached PTO-2038.

For the following reasons, Appellant respectfully submits that the final rejection of each of Claims 1, 4, 5, 12-16 and 18-21 in this application is in error, and therefore respectfully requests reversal of the rejections.

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**I. Real Party in Interest**

The real party in interest is Ajinomoto Co., Inc, a corporation of Japan.

**II. Related Appeals and Interferences**

There are no related appeals or interferences.

**III. Status of Claims**

Claims 1, 4, 5, 8-16, and 18-21 are pending. Claims 1, 4, 5, 12-16 and 18-21 are rejected in the Office Action dated October 16, 2008, and are on appeal. Claims 8-11 are withdrawn from consideration as being drawn to a non-elected invention. Claims 2, 3, 6, 7 and 17 are cancelled.

**IV. Status of Amendments**

All amendments to the claims have been entered.

**V. Summary of Claimed Subject Matter**

Claim 1: An isolated coryneform bacterium having L-glutamine-producing ability, wherein said bacterium has been modified by disrupting or mutating a glutaminase gene on the chromosome so that the glutaminase activity of the bacterium is reduced to 0.1 U/mg of cellular protein or less, wherein said glutaminase gene is selected from the group consisting of a DNA comprising the DNA sequence of SEQ ID NO:1, and a DNA which is able to hybridize with the DNA of SEQ ID NO: 1 under stringent conditions of 1 X SSC, 0.1% SDS, at 60°, and is 95% or more homologous to SEQ ID NO: 1. [see pages 5-6, 8-13, and original claim 3].

**VI. Grounds of Rejection to Be Reviewed on Appeal**

A. Whether Claims 1, 4-5, 12-16, and 18-21 are unpatentable under 35 U.S.C. § 112, 2<sup>nd</sup> paragraph, for being indefinite.

B. Whether Claims 1, 4-5, 12-16, 18-19 and 21 are unpatentable under 35 U.S.C. § 112, 1<sup>st</sup> paragraph, for lack of an adequate written description.

C. Whether Claims 1, 4-5, 12-16, and 18-21 are unpatentable under 35 U.S.C. § 112, 1<sup>st</sup> paragraph, for failing to comply with the scope of enablement request.

D. Whether Claims 1, 4-5, 13-16, and 19-21 are unpatentable under 35 U.S.C. § 103(a) over Nakamura *et al.* in view of Pompejus *et al.*, Jakoby *et al.*, and further in view of Duran *et al.*, as evidenced by Nakagawa *et al.*

## VII. Argument

### A. Legal Standard

35 U.S.C. § 112, 2<sup>nd</sup> paragraph, mandates that a patent specification must “conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.” This determination is a question of law, which “focuses on whether the claims, as interpreted in view of the written description, adequately perform their function of notifying the public of the [scope of the] patentee’s right to exclude.” *Honeywell International, Inc. v. Int’l Trade Comm’n*, 341 F.3d 1332, 1339 (Fed. Cir. 2003) (citation omitted). Thus, if a claim term may be given more than one definition, but the intrinsic record does not compel a narrowing of the claim language to any of the definitions, the claim term is indefinite. *Id.* at 1338 (finding claims to be invalid after noting “[t]he intrinsic record does not compel a narrowing of the claim language to any one of the possible definitions. Finally, the test for definiteness under 35 U.S.C. § 112, second paragraph, is whether “those skilled in the art would understand what is claimed when the claim is read in light of the specification.” *Orthokinetics, Inc. v. Safety Travel Chairs, Inc.*, 806 F.2d 1565, 1576 (Fed. Cir. 1986).

Because the written description requirement is separate and distinct from the enablement requirement, *Vas-Cath, Inc. v. Mahurkar*, 935 F.2d 1555, 1562 (Fed. Cir. 1991), these issues are addressed separately.

A claimed invention is unpatentable due to the lack of a written description if the specification fails to “clearly convey the information that an applicant has invented the subject matter which is claimed”, *In re Barker* 559 F.2d 588, 592 (CCPA 1977), or if possession of what

applicant claims as the invention is not put in the public domain. See *Regents of the University of California v. Eli Lilly*, 119 F.3d 1559, 1566 (Fed. Cir. 1997), *cert. denied*, 523 U.S. 1089 (1998). To satisfy the written description requirement, possession must be shown; however possession alone does not cure the lack of a written description. *Enzo Biochem, Inc. v. Gen-Probe, Inc.*, 296 F.3d 1316, 1330 (Fed. Cir. 2002). For a claimed genus, the written description requirement may be satisfied through sufficient description of a representative number of species by disclosure of relevant identifying characteristics, *i.e.*, structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or a combination of the above. See *Eli Lilly*, 119 F.3d at 1568. Description of a representative number of species does not require the description to be of such specificity that it would provide individual support for each species that the genus embraces. See M.P.E.P § 2163, II, A, 3, ii.

A claimed invention is unpatentable due to a non-enabling disclosure if the specification fails to describe how to make and how to use the invention. See 35 U.S.C. § 112, 1<sup>st</sup> paragraph. The test for this standard is whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with information known in the art, without undue experimentation. *United States v. Teletronics, Inc.*, 857 F.2d 778, 785 (Fed. Cir. 1988). The seminal case in determining if a claim meets this standard is *In re Wands*, 858 F.2d 731, 737 (Fed. Cir. 1988), which promulgated a series of factors, set forth throughout the prosecution (*see*, for example, the First Office Action of December 26, 2007, page 8 and the Final Office Action of September 4, 2008, page 5) to be considered when determining whether there is sufficient evidence to support a determination that a disclosure does not satisfy the enablement requirement and whether any necessary experimentation is ‘undue.’

Claimed subject matter is obvious in light of the prior art if it would have been obvious to one of ordinary skill in the relevant art at the time the invention was made. See 35 U.S.C. § 103(a). In considering the entire prior art in the relevant field, the claimed subject matter is obvious if the prior art “would have suggested to one of ordinary skill in the art that this

[invention should be made] and would have a reasonable likelihood of success.” *In re Dow Chemical Co.*, 837 F.2d 469, 473 (Fed. Cir. 1988).

Obviousness can be shown either directly by demonstrating the technical motivation to combine the prior art, *Life Technologies, Inc. v. Clontech Laboratories, Inc.*, 224 F.3d 1320, 1326 (Fed. Cir. 2000), or indirectly through “secondary considerations” after the claimed subject matter was invented, *Custom Accessories, Inc. v. Jeffrey-Allan Industries, Inc.*, 807 F.2d 955, 960 (Fed. Cir. 1986). To show the motivation to combine prior art, it is not enough to simply identify different references that might be combined in hindsight; showing obviousness requires showing a motivation to combine the pieces. *Velander v. Garner*, 348 F.3d 1359, 1363 (Fed. Cir. 2003). That motivation might come from a reference or from the knowledge of an artisan of ordinary skill. The level of ordinary skill in an art is based on a number of factors, including the educational level of the inventor, the type of problems encountered in the art, prior solutions to those problems, and the speed of innovation in the art. *Ruiz v. A.B. Chance Co.*, 234 F.3d 654, 666-67 (Fed. Cir. 2000).

“Secondary considerations” focus on how the invention was received in the market; a very successful or surprising invention is probably not obvious. See *Custom Accessories, Inc.*, 807 F.2d at 960. The “secondary considerations” considered by the courts include: commercial success, copying of the invention by others, licensing of the invention, evidence of a long-felt need for the invention, skepticism by skilled artisans that the claimed invention could be achieved, prior failures of others to achieve the same result, and unexpected results. *Id.*; *Pentec, Inc. v. Graphic Controls Corp.*, 776 F.2d 309, 316 (Fed. Cir. 1985). No secondary consideration is required for an invention to be non-obvious, but a court may use evidence of secondary considerations in its determination of obviousness (or non-obviousness). *Custom Accessories, Inc.*, 807 F.2d at 960.

Finally, the “teaching, suggestion, motivation” test (the “TSM” test) which is often used to prove obviousness was revised somewhat in a recent Supreme Court decision. See *KSR International Co. v. Teleflex Inc. et al.*, No. 04-1350, slip op. at 16 (S.Ct., April 30, 2007). This test, as applied in this case, was determined to be too rigidly applied. The Supreme Court said: “There is no necessary inconsistency between the idea underlying the TSM test and the Graham

analysis. But when a court transforms the general principle into a rigid rule that limits the obviousness inquiry . . . it errs.” The Federal Circuit erred by (1) looking only at the problem the patentee was trying to solve, (2) assuming that a person of ordinary skill attempting to solve a problem will be led only to those elements of prior art designed to solve the same problem, (3) concluding that a patent claim cannot be proved obvious by showing the combination of elements was “obvious to try,” and (4) applying a rigid rule to prevent hindsight that denied factfinders “recourse to common sense.”

In its broadest sense, the *KSR* decision broadens the inquiry that must be made when proving an invention is obvious over prior art. Before this decision, when combining references, an Examiner must show a motivation by the person of skill in the art to combine the teachings of the different references. In the decision, the Supreme Court states that such a showing is not required, but only a “reason” to combine the teachings should be shown. This reason can be based upon logic, common sense, and/or the knowledge in the art. The Court stated that when familiar elements are combined according to known methods, the invention is likely to be obvious when it does no more than yield predictable results.” *KSR*, *slip op.* at 12.

Also, the Court explained that when the prior art elements work together in an unexpected and fruitful manner, the invention is not obvious. *Id.* (citing *United States v. Adams*, 383 U.S. 39, 40 (1966)). If, however, the combination of old elements does no more than they would in separate, sequential operation, even though the combination might perform a useful function, the combination is likely obvious. *Id.* at 13 (citing *Anderson's-Black Rock, Inc. v. Pavement Salvage Co.*, 396 U.S. 57 (1969)).

These statements regarding expectation are not so different from the current test for proving obviousness, however, the Court does broaden the analysis in its discussion of expanding of the “teaching, suggestion, or motivation” test. As stated above, a specific or rigid motivation is no longer required, but only a logical reason for combining prior art. This standard is less stringent.

*B. The rejection of Claims 1, 4, 5, 12-16, and 18-21 under 35 U.S.C. §112, 2<sup>nd</sup> paragraph is in error*

In the Office Action dated October 16, 2008, beginning at page 2, Claims 1, 4-5, 12-16, and 18-21 were rejected under 35 U.S.C. § 112, second paragraph, as reciting subject matters that allegedly are indefinite. Applicant respectfully requests reconsideration of this rejection.

The Examiner has stated that the phrase “95% or more homologous to SEQ ID NO:X” is “unclear and confusing in the absence of a definition providing the intended meaning of the term or the intended parameters required to determine the required homology value.” Such ignores established precedent in Group 1600, and particularly precedent set by other Examiners and the Supervisor *in the Examiner’s own art unit*. Specifically, Appellants have numerous patents issued from this art unit directed to similar-type claims, for example, bacteria which have been modified to increase or decrease expression of a known gene and its homologs within 90-95% homology for the purpose of producing a substance, usually an amino acid, wherein the supporting disclosure describing determination of homology is the same. Recent patents of Appellants issuing from art unit 1652 having homology language for defining similar genes or proteins in a claim include **7,300,786; 7,186,531; 7,160,704; and 6,893,852**. Furthermore, the following patents have homology language in the claims, but were issued from a different group 1600 art unit, including one (the ‘998 patent) which was issued on an order from the Board of Patent Appeals and Interferences: **7,312,058, 7,244,569, 7,090,998, and 6,905,819**. Therefore, it is clear that the use of homology language to define similar sequences in a claim is an accepted method for claiming variants to a claimed amino acid or gene sequence.

A major reason why such language is universally accepted as being clear and definite lies not so much in the patent application’s specification, but in the standardized and well-practiced methods for determining homology between sequences known to those of skill in the art. It is well known in the art how to calculate homology using the computer program BLAST. The parameters used in this program, although they can be varied, have been standardized, and so determination of homology between sequences is commonly and routinely practiced in the art with consistent and meaningful results recognizable to those of skill in the art. Therefore, the computer program BLAST has standardized the methods for comparing sequences and



establishing a homology, and is commonly and routinely used for such.

Specific statements in the Office Action of October 16, 2008 will now be addressed. First, the reasons presented on page 3 detailing why claims 12 and 18 are rejected under this section is entirely unclear. The objectionable language is not present in claims 12 and 18, and the Examiner's statement is correct that the scope of claims 12 and 18 are a subgroup of the entire group of genes encoding the polypeptide of SEQ ID NO:2. However, the genus of genes in claims 12 and 18 is defined as those which encode the amino acid sequence of SEQ ID NO: 2. This is a very definite group of genes, and essentially nullifies the 95% homology language of claim. In proper claim drafting and interpreting, claims 12 and 18 further limit claim 1, and this further limitation further defines the genus of possible genes to those genes which encode a specific sequence. This is definite language, and the scope of the claim is perfectly clear. The rejection of claims 12 and 18 for the presence of the language "95% homologous" is simply wrong, and should be withdrawn.

On page 4, the Examiner states that the reason for the rejection is that one of ordinary skill in the art cannot determine the scope of the term "95% homologous", and then presents 3 distinct arguments to support this reason. First, the Examiner states that there is no indication in the specification that the intended meaning of the term "homology" is "identity". This is because Appellants mean to claim "95% homologous" and not "95% identity". Appellants are well aware that the term "homology" and "identity" are not equivalent, and the specification clearly reflects this awareness. Appellants chose to claim "95% homologous", and not "95% identity", and for the Examiner to construe the claim any other way is impermissible. Secondly, the Examiner states that "it is clear from Applicant's response that the intended meaning of the term is not identity". This is because Appellants intend "homology". The claims recite "homology", and not "identity", so why would a person assume that Appellants might mean identity? The specification and the knowledge in the art clearly define how to determine homology between two sequences, so Appellants intention is clear and defined. Thirdly, the Examiner states that "the specification does not provide the specific parameters/methods intended in the calculation of sequence homology". This is just simply incorrect, and the Examiner must also look beyond the specification and to the knowledge in the art for the definition of this term. Scientists have been

determining homologous sequences for decades, and the Examiner acknowledges at the top of page 4 that the calculation of sequence homology takes into consideration the type of mismatches, and these can contribute to the calculation. Such calculations are routine and, although they may give varying results, the ordinarily skilled art worker has been making such calculations routinely for so long that such variances can be accounted for and recognized. Just because a determination may be variable does not mean it is undefined, particularly when the variables have been recognized and the reasons for obtaining such variables are also well-known.

For these reasons, the use of the homology language in the claims is clear and definite as one of ordinary skill in the art would be able to determine the sequences which fall within the 95% homology limitation to the recited SEQ ID NO.. The rejection under 35 U.S.C. 112, 2<sup>nd</sup> should be withdrawn. For at least the foregoing reasons, Appellants respectfully submit that Claims 1, 4-5, 12-16, and 18-21 fully comply with 35 U.S.C. § 112, second paragraph, and therefore respectfully requests withdrawal of the rejection thereof under 35 U.S.C. § 112.

C. The rejection of Claims 1, 4, 5, 12-16, 18, 19, and 21 under 35 U.S.C. §112, 1<sup>st</sup> paragraph, written description, is in error

In the Office Action dated October 16, 2008, beginning at page 5, Claims 1, 4-5, 12-16, 18-19 and 21 were rejected under 35 U.S.C. § 112, first paragraph, as reciting subject matters that allegedly fail to comply with the written description requirement. Applicant respectfully requests reconsideration of this rejection.

The Examiner acknowledges on page 6 that the issue of indefiniteness due to the recitation of “95% or more homologous to SEQ ID NO: 1” was not considered in making this specific rejection. Rather, the Examiner states that the issue in this written description rejection is that “the claims require a precise reduction in glutaminase activity to a specific level....and a precise ratio of glutamine synthetase activity to glutaminase activity ratio obtained by any means.” Appellants respectfully disagree with the Examiner’s assessment and interpretation of the claims. First, “a precise reduction” is not required by the claims, but a reduction to *any level* below 0.1 U/mg. The claim does not require one to achieve exactly 0.1 U/mg, but the claim requires only that the gene is disrupted or mutated so that the level falls below this activity

number. Also, regarding the ratio of activities in claims 4 and 15, again, the claims do not require the skilled art worker to achieve *exactly* 1/2 ratio of glutaminase to glutamine synthetase activity, but only that the ratio should be below this number. Finally, the Examiner states that these values for activity and activity ratio can be obtained by any means. Again, this is simply incorrect. The claim requires that the glutaminase gene is disrupted or mutated to reduce its activity below the stated level, and to obtain the claimed ratio. The disruption and/or mutation of genes are well-known procedures in the art, and the teachings of the specification combined with these well-known methods clearly demonstrate that the claimed invention is adequately described.

Furthermore, Appellants presented an alignment of the protein sequences of glutaminase (gls) (Exhibit A) in the July 31, 2007 response. One of ordinary skill in the art would clearly recognize which regions are important for the enzymatic activities of the proteins from, at least, this alignment, and would be able to reduce the activities of the proteins by introducing amino acid mutations at such regions, and hence the claims are fully and adequately described. Furthermore, one of ordinary skill in the art would be able to readily ascertain an expression regulatory sequence of the recited glutaminase gene on the chromosome of a coryneform bacterium based on the sequence information for glutaminase genes, and would be able to mutate or disrupt the expression regulatory sequence of the recited glutaminase gene. That is, one of ordinary skill in the art would be able to obtain a coryneform bacterium in which glutaminase activity is reduced to *0.1U/mg of cellular protein or any level below this activity level*, by mutating or disrupting the recited glutaminase gene on the chromosome of a coryneform bacterium and/or by mutating or disrupting an expression regulatory sequence of the recited glutaminase gene, based on the known sequence information for glutaminase genes, the teaching of the specification, and the knowledge and level of skill in this art. No undue experimentation is required to determine such information, and the invention is fully and adequately described, particularly in view of the knowledge in the prior art regarding the sequences.

Also, as is evidenced by the alignment of the nucleotide sequences of glutamine synthetase gene (gin) from *B. flavum* (SEQ IDNO: 3), *C. glutamicum*, *C. efficiens*, and *Mycobacterium tuberculosis*, the gln genes are highly conserved (Exhibit B in the July 31, 2007

response). Therefore, one of ordinary skill in the art would reasonably understand that a DNA sequence which has 95% or more homology to SEQ ID NO: 3 would naturally encode a protein having glutamine synthetase activity. Thus, one of ordinary skill in the art would reasonably be able to ascertain structures of the recited homologous gene which encodes a glutamine synthetase. Also, claims 5 and 16 have been amended to restrict the means for increasing glutamine synthetase activity to “by increasing the copy number of glutamine synthetase gene” or “by replacing promoter region of glutamine synthetase gene with a stronger promoter”. These means are fully supported by the present specification (see page 17, lines 15—21) Therefore, one of ordinary skill in the art is believed to reasonably understand that by increasing the copy number of the recited glutamine synthetase gene or by replacing promoter region of the recited glutamine synthetase gene with a stronger promoter, glutamine synthetase activity would be enhanced.

For at least the foregoing reasons, Appellants respectfully submit that Claims 1, 4-5, 12-16, 18-19 and 21 fully comply with 35 U.S.C. § 112, first paragraph, and therefore respectfully requests withdrawal of the rejection thereof under 35 U.S.C. § 112.

*D. The rejection of Claims 1, 4, 5, 12-16, and 18-21 under 35 U.S.C. §112, 1<sup>st</sup> paragraph, enablement, is in error*

In the Office Action dated October 16, 2008, beginning at page 8, Claims 1, 4-5, 12-16, and 18-21 were rejected under 35 U.S.C. § 112, first paragraph, as reciting subject matters that allegedly fail to comply with the scope of enablement requirement. Applicant respectfully requests reconsideration of this rejection.

Similar to the above rejection for lack of written description, the Examiner states on page 9 of the October 16, 2008 Office Action that while the complete inactivation of the gene is enabled, the fact that “the claims require a precise reduction in glutaminase activity to a specific level....and a precise ratio of glutamine synthetase activity to glutaminase activity ratio obtained by any means” results in a scope which is not enabled. Appellants respectfully disagree with the Examiner’s assessment and interpretation of the claims. First, “a precise reduction” is not

required by the claims, but a reduction to *any level* below 0.1 U/mg. The claim does not require one to achieve exactly 0.1 U/mg, but the claim requires only that the gene is disrupted or mutated so that the level falls below this activity number. Also, regarding the ratio of activities in claims 4 and 15, again the claims do not require the skilled art worker to achieve *exactly* 1/2 ratio of glutaminase to glutamine synthetase activity, but only that the ratio should be below this number. Finally, the Examiner states that these values for activity and activity ratio can be obtained by any means. Again, this is simply incorrect. The claim requires that the glutaminase gene is disrupted or mutated to reduce its activity below the stated level, and to obtain the claimed ratio. The disruption and/or mutation of genes is a well-known procedure in the art, and the specification combined with these well-known methods clearly demonstrate that the claimed invention is adequately described. Furthermore, it is illogical that the Examiner finds the end point of 0 U/mg activity enabled, but the range from 0.1 to just above 0 U/mg is not enabled. Finally, inoperable embodiments are permitted to be encompassed by the claims, and exemplification of every embodiment is not required to satisfy 35 U.S.C. §112, 1<sup>st</sup> paragraph (*Altas Powder Co. v. E.I. duPont de Nemours and Co.*, 750 F.2d 1569, 1577 (Fed. Cir. 1984)). Therefore, because the specification does not describe or exemplify exactly how to obtain a disrupted or mutated glutaminase gene which encodes a glutaminase protein with an activity of 0.098 U/mg, for example, does not mean the claims are not enabled.

Furthermore, and similar to the above arguments, Appellants presented an alignment of the protein sequences of glutaminase (gls) (Exhibit A) in the July 31, 2007 response. One of ordinary skill in the art would clearly recognize which regions are important for the enzymatic activities of the proteins from at least this alignment, and would be able to reduce the activities of the proteins by introducing amino acid mutations at such regions, and hence the scope of the claims is fully and adequately enabled. Furthermore, one of ordinary skill in the art would be able to readily ascertain expression regulatory sequences of the recited glutaminase gene on the chromosome of a coryneform bacterium based on the sequence information for glutaminase genes, and would be able to mutate or disrupt the expression regulatory sequence of the recited glutaminase gene. That is, one of ordinary skill in the art would be able to obtain a coryneform bacterium in which glutaminase activity is reduced to *0.1U/mg of cellular protein or any level*

*below this activity level*, by mutating or disrupting the recited glutaminase gene on the chromosome of a coryneform bacterium and/or by mutating or disrupting expression regulatory sequence of the recited glutaminase gene, based on the known sequence information for glutaminase genes, the teaching of the specification, and the knowledge and level of skill in this art. No undue experimentation is required to determine such information, particularly in view of the knowledge in the prior art regarding the sequences.

Also, as is evidenced by the alignment of the nucleotide sequences of glutamine synthetase gene (gln) from *B. flavum* (SEQ IDNO: 3) (Exhibit B), *C. glutamicum*, *C. efficiens*, and *Mycobacterium tuberculosis*, the gln genes are highly conserved (Exhibit B in the July 31, 2007 response). Therefore, one of ordinary skill in the art would reasonably understand that a DNA sequence which has 95% or more homology to SEQ ID NO: 3 would naturally encode a protein having glutamine synthetase activity. Thus, one of ordinary skill in the art would reasonably be able to ascertain structures of the recited homologous gene which encodes a glutamine synthetase. Also, claims 5 and 16 have been amended to restrict the means for increasing glutamine synthetase activity to “by increasing the copy number of glutamine synthetase gene” or “by replacing promoter region of glutamine synthetase gene with a stronger promoter”. These means are fully supported by the present specification (see page 17, lines 15—21) Therefore, one of ordinary skill in the art would reasonably understand that by increasing the copy number of the recited glutamine synthetase gene or by replacing promoter region of the recited glutamine synthetase gene with a stronger promoter, glutamine synthetase activity would be enhanced.

For at least the foregoing reasons, Appellants respectfully submit that Claims 1, 4-5, 12-16 and 18-21 fully comply with 35 U.S.C. § 112, first paragraph, and therefore respectfully requests withdrawal of the rejection thereof under 35 U.S.C. § 112.

E. The rejection of Claims 1, 4, 5, 13-16, and 19-21 under 35 U.S.C. §103

In the Office Action dated October 16, 2008, beginning at page 11, Claims 1, 4-5, 13-16, and 19-21 were rejected under 35 U.S.C. § 103(a), as reciting subject matters that allegedly are

obvious, and therefore allegedly unpatentable, over the disclosure of Nakamura *et al.* (hereinafter “Nakamura”) in view of the disclosure of Pompejus *et al.* (hereinafter “Pompejus”), Jakoby *et al.* (hereinafter “Jakoby”), and further in view of the disclosure of Duran *et al.* (hereinafter “Duran”), as evidenced by the disclosure of Nakagawa *et al.* (hereinafter “Nakagawa”). Applicant respectfully requests reconsideration of this rejection.

The claims are directed to a coryneform bacterium which has been modified by disrupting or mutating the glutaminase gene so that the activity is reduced to a certain level. The glutaminase gene is specifically defined by reference to its DNA sequence and certain defined variants thereof. Nakamura is cited for teaching glutamine production by fermentation of a coryneform bacterium, which is typically performed in the presence of glucose in the medium. However, there is no teaching in Nakamura of disabling the glutaminase gene, nor any suggestion for doing so. Pompejus teach the genes encoding the glutaminase and glutamine synthetase from *C. glutamicum*, which Appellants do not dispute. Jakoby is cited for teaching a *C. glutamicum* glutamine synthetase gene and its corresponding glutamine synthetase, which is 99.1% identical to the polypeptide of SEQ ID NO:3. Finally, Duran teach that the glutaminase-deficient mutant strain of *Rhizobium etli* (LM16) demonstrates an increase in the intracellular glutamine level when the mutant strain was cultivated in the presence of glutamine as the carbon source (Table 1). Nakagawa is cited as further evidence of the structure of the *C. glutamicum* glutaminase gene.

One of ordinary skill in the art would not be motivated or have any reason to combine the teachings of these references. Specifically, Duran only teaches uptake of glutamine added to the medium by the bacterial cells, and does not show production of glutamine by the LM16 strain in the culture medium. Specifically, as shown in Table 1 of Duran, the intracellular glutamate level in the LM16 mutant is low as compared to that in the wild-type strain when the mutant is cultivated in medium containing ammonium and succinate as growth substrates (see the 3<sup>rd</sup> column from the top). In general, it is known that glutamine is synthesized from glutamate and ammonium via the catalytic effect of glutamine synthetase. Therefore, one of ordinary skill in the art would know that the production of glutamine decreases when the intracellular glutamate concentration decreases.

In Figure 3 of Duran, the  $^{14}\text{CO}_2$  which is released from  $[\text{U-}^{14}\text{C}]$  glutamine by the LM16 mutant (which lacks glutaminase activity) and wild-type strain are shown, when these cells cultivated in medium containing glutamine and succinate. It is clearly shown in Fig.3 that the amount of released  $^{14}\text{CO}_2$  from the LM16 mutant and wild-type strain is almost the same. This means that glutaminase is hardly involved in degradation of glutamine in LM16 and the wild-type. Therefore, one of ordinary skill in the art would not conclude from reading Duran that knocking out glutaminase would result in increased glutamine productivity, since glutaminase is clearly not involved, or involved very minimally, in the degradation of glutamine.

Furthermore, Duran uses *Rhizobium etli*, which is known as one of the rhizobial strains. It is known that the rhizobial strains use nitrogen assimilation similar to that used and studied in plants. Furthermore, it is also known that the rhizobial strains have a particular nitrogen metabolism pathway as compared with other types of bacteria. The paper of Duran issued in 1995. However, later review papers which describe the nitrogen metabolism of other types of bacteria such as *Corynebacterium* and *E.coli* do not describe the glutaminase as described in Duran. Therefore, one of ordinary skill in the art would consider from a reading of Duran that glutaminase does NOT play an important role in nitrogen metabolism of *C. glutamicum* or *E.coli*. Therefore, one of ordinary skill in the art would not be motivated to decrease glutaminase activity in order to increase glutamine productivity.

The phenomenon that "intracellular glutamine levels are increased when glutamine is used as a carbon source" is likely because glutamine, which has been imported into the bacterial cells, is not degraded in the absence of glutaminase. Furthermore, when succinate and glutamine were simultaneously added to the medium, intracellular glutamine levels decreased as compared to when glutamine was used as the sole carbon source. This strain is not believed to have the ability to produce glutamine. Most notably, one of ordinary skill in the art would have reasonably concluded that the glutamine level in bacterial cells would *decrease*, based on the teachings of Nakamura combined with the teaching of Duran, Jakoby, and Pompejus. The effect realized in the claimed invention, and reflected in the claims, is the opposite, in that an increase in glutamine production is seen with the decrease in glutaminase activity.

On the other hand, the bacterium as claimed in the present application is a coryneform



bacterium which has L-glutamine-producing ability. As described in the specification of the present application (see page 6, lines 17-19), "L-glutamine-producing ability" means an ability of the bacterium to accumulate L-glutamine in a medium when the bacterium is cultivated in the medium. Such bacterium having L-glutamine-producing ability is believed not to be disclosed or suggested by Duran, and is certainly not disclosed in any of the other cited references. The combination of their various teachings does not cure this fatal deficiency.

Furthermore, the *Rhizobium* bacterium as taught by Duran is known to have an enzyme which catalyzes the formation of 2 molecules of glutamic acid from 1 molecule of glutamine and 1 molecule of  $\alpha$ -ketoglutarate ( $\alpha$ KG) in the presence of NADPH. This enzyme is called GOGAT, and is also present in coryneform bacterium. GOGAT does not function in the absence of  $\alpha$ KG. As is seen in Table 1 of Duran, glutamine accumulates in the cells when glutamine is added as the sole carbon source. This is probably because when glutamine is used as the sole carbon source,  $\alpha$ KG is not produced in the cells and GOGAT does not work. On the other hand, simultaneous addition of succinate and glutamine lead to a marked decrease in intracellular glutamine levels as compared to when glutamine is present as the sole carbon source. This is probably because GOGAT works to degrade glutamine in the presence of succinate. The reason why GOGAT works to degrade glutamine in the presence of succinate is that when succinate is added,  $\alpha$ KG is produced via the TCA cycle. Also, because pyruvate is formed from succinate, and then acetyl CoA is formed from pyruvate, the TCA cycle can use the produced acetyl CoA. It is well known that glutamine fermentation is typically performed in the presence of glucose. During such fermentation,  $\alpha$ KG accumulates in the fermentation broth. Therefore, a sufficient amount of  $\alpha$ KG is present in the cells when the cells are cultivated in the presence of glucose. Also, producing  $\alpha$ KG from glucose ( $C6 \rightarrow C5$ ) is more efficient than producing  $\alpha$ KG from succinate ( $C4 \rightarrow C5$ ), because the former does not need an extra metabolic pathway. Therefore, GOGAT would be expected to have a greater contribution to the degradation of glutamine when glucose is added to the medium, as compared to when succinate is added to the medium.

In view of the above, Duran clearly do not teach or suggest an increased glutamine level in a glutaminase-deficient bacterium when glucose is present in the medium. In fact, Duran actually teaches away from the claimed invention, that in the presence of glucose, glutamine

levels in such bacterium decrease.

Thus, there is no reason or motivation to combine Nakamura, Duran, Jakoby, and/or Pompejus and from their combined teachings, arrive at a glutamine-producing bacterium with reduced glutaminase activity. Furthermore, through no combination of the cited references is it obvious for one of ordinary skill in the art to modify a coryneform bacterium to reduce intracellular glutaminase activity with the expectation of obtaining a bacterium with enhanced L-glutamine producing ability.

For at least the foregoing reasons, Applicants respectfully submit that the subject matters of Claims 1, 4-5, 13-16, and 19-21, each taken as a whole, would not have been obvious to one of ordinary skill in the art at the time of Appellant's invention, are therefore not unpatentable under 35 U.S.C. § 103(a), and therefore respectfully requests withdrawal of the rejection thereof under 35 U.S.C. § 103(a).

**VIII. Conclusion**

For at least the reasons presented herein, each of the subject matters of Claims 1, 4-5, 8-16 and 18-21 taken as a whole, are patentable in view of the written description and enablement requirements of 35 U.S.C. §112, 1<sup>st</sup> paragraph, are definite in view of the requirements of 35 U.S.C. §112, 2<sup>nd</sup> paragraph, and are patentable over the disclosures of Nakamura, Pompejus, Jakoby, Duran, and Nakagawa, in view of 35 U.S.C. §103(a). Accordingly, the rejections of each of Claims 1, 4, 5, 12-16 and 18-21 are reversible errors.

Respectfully submitted,

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**APPENDIX A: CLAIMS ON APPEAL**

1. An isolated coryneform bacterium having L-glutamine-producing ability, wherein said bacterium has been modified by disrupting or mutating a glutaminase gene on the chromosome so that the glutaminase activity of the bacterium is reduced to 0.1 U/mg of cellular protein or less, wherein said glutaminase gene is selected from the group consisting of:

- a) a DNA comprising the DNA sequence of SEQ ID NO: 1, and
- b) a DNA which is able to hybridize with the DNA of SEQ ID NO: 1 under stringent conditions of 1 X SSC, 0.1% SDS, at 60°C, and is 95% or more homologous to SEQ ID NO: 1.

4. The bacterium of claim 1, wherein said glutaminase activity is 1/2 or less than glutamine synthetase activity when measured as activity per unit weight of cellular proteins.

5. The bacterium of claim 1, which is further modified by increasing the expression of a glutamine synthetase gene by increasing the copy number of said glutamine synthetase gene or by replacing a promoter region of said glutamine synthetase gene with a stronger promoter so that said glutamine synthetase activity of the bacterium is enhanced, wherein said glutamine synthetase gene is selected from the group consisting of:

- c) a DNA comprising the DNA sequence of SEQ ID NO: 3, and
- d) a DNA which is able to hybridize with the DNA of SEQ ID NO: 3 under stringent conditions of 1 X SSC, 0.1% SDS, at 60°C, and is 95% or more homologous to SEQ ID NO: 3, and which encodes a protein which has glutamine synthetase activity.

12. The bacterium of claim 1, wherein said glutaminase gene encodes a protein comprising the amino acid sequence of SEQ ID NO: 2.

13. The bacterium of claim 5, wherein said stronger promoter is selected from the group consisting of the lac promoter, trp promoter, and trc promoter.

14. The bacterium of claim 1, wherein the glutaminase activity of the bacterium is reduced to 0.01 U/mg of cellular protein or less.

15. The bacterium of claim 14, wherein said glutaminase activity is 1/2 or less than glutamine synthetase activity when measured as activity per unit weight of cellular proteins.

16. The bacterium of claim 14, which is further modified by increasing the expression of a glutamine synthetase gene by increasing the copy number of said glutamine synthetase gene or by replacing a promoter region of said glutamine synthetase gene with a stronger promoter so that said glutamine synthetase activity of the bacterium is enhanced, wherein said glutamine synthetase gene is selected from the group consisting of:

- c) a DNA comprising the DNA sequence of SEQ ID NO: 3, and
- d) a DNA which is able to hybridize with the DNA of SEQ ID NO: 3 under stringent conditions of 1 X SSC, 0.1% SDS, at 60°C, and is 95% or more homologous to SEQ ID NO: 3, and which encodes a protein which has glutamine synthetase activity.

18. The bacterium of claim 14, wherein said glutaminase gene encodes a protein comprising the amino acid sequence of SEQ ID NO: 2.

19. The bacterium of claim 16, wherein said stronger promoter is selected from the group consisting of the lac promoter, trp promoter, or trc promoter.

20. The bacterium of claim 1, wherein said glutaminase activity is reduced by disrupting a glutaminase gene on the chromosome.

21. The bacterium of claim 1, wherein said glutaminase activity is reduced by mutating a glutaminase gene on the chromosome.

## APPENDIX B: EVIDENCE

Exhibit A: Alignment of the protein sequences of glutaminase (gls).

Exhibit B: Alignment of the nucleotide sequences of glutamine synthetase gene (gin) from *B.*

*flavum* (SEQ IDNO: 3).

**APPENDIX C: RELATED PROCEEDINGS**

None.